SUPPLEMENTAL MATERIAL

Methods:

Determination of cardiac function: Cardiac function was determined by echocardiography as well as left ventricular catheterization. Determination of cardiac function occurred 2 weeks after mini-osmotic pump implantation, prior to thoracotomy, as described previously.

Histological analysis: Hearts were fixed with 4% paraformaldehyde, embedded in paraffin, and either coronally (for myocyte/fibrosis ratio in left ventricular free wall) or transversely(for all other assays) sectioned (6 μm thick). Five sections from each heart were mounted upon glass slides, and stained with Masson trichrome (for fibrosis determination), wheat germ agglutinin (for myocyte size), antibody against CD31 (for capillary density), or antibody against α-smooth muscle actin (α-SMA, for arteriolar density). Slides were examined with an Olympus IX51 microscope, and 5 images from each slide were captured by a Q-Imaging camera controlled by IP Lab 4.0 software. Myocyte/fibrosis ratio (left ventricular free wall), cardiac collagen deposit (remote non-infract area), and capillary/arteriolar density (infarct border zone) were determined as previously reported[1, 2]. Results from all slides obtained in the same heart were averaged, and counted as n=1.

Western blot analysis: Proteins of interest were separated on SDS-PAGE gels, transferred to PVDF membranes, and incubated with appropriate primary antibodies followed by horseradish peroxidase-conjugated secondary antibody. Blots were developed via SupersignalChemiluminescence detection kit (Pierce, Rockford, Ill). Bands were visualized with a Kodak Image Station 4000 (Rochester, NY).

Tube formation assay: 100 μl of Matrigel (growth factor reduced, BD Biosciences) was added to each well of a 48-well plate, and polymerized at 37°C for 1 hour. HUVECs (1×10^4) were seeded onto Matrigel, in endothelial cell basal medium-2 with EGM-2 Bullet Kit. After 1 hour culture, gCTRP3 (3 μg/ml) or full length CTRP3 (30 μg/ml) was added. For conditioned medium experiments, endothelial cell basal medium-2 was replaced with cardiomyocyte-conditioned medium after 1 hour culture. After an additional 6 hour-culture, tube length was quantified via IP Lab 4.0 image analysis software.

Assessment of cardiomyocyte apoptosis: Cardiomyocyte apoptosis was determined via TUNEL staining and caspase-3 activity, as reported previously[3].

Measurements of endotoxin activity: The endotoxin activities of globular CTRP3 preparations were determined using the LAL assay kit (catalog No.50–648U, BioWhittaker, Walkersville, MD) according to the manufacturer's recommendation.

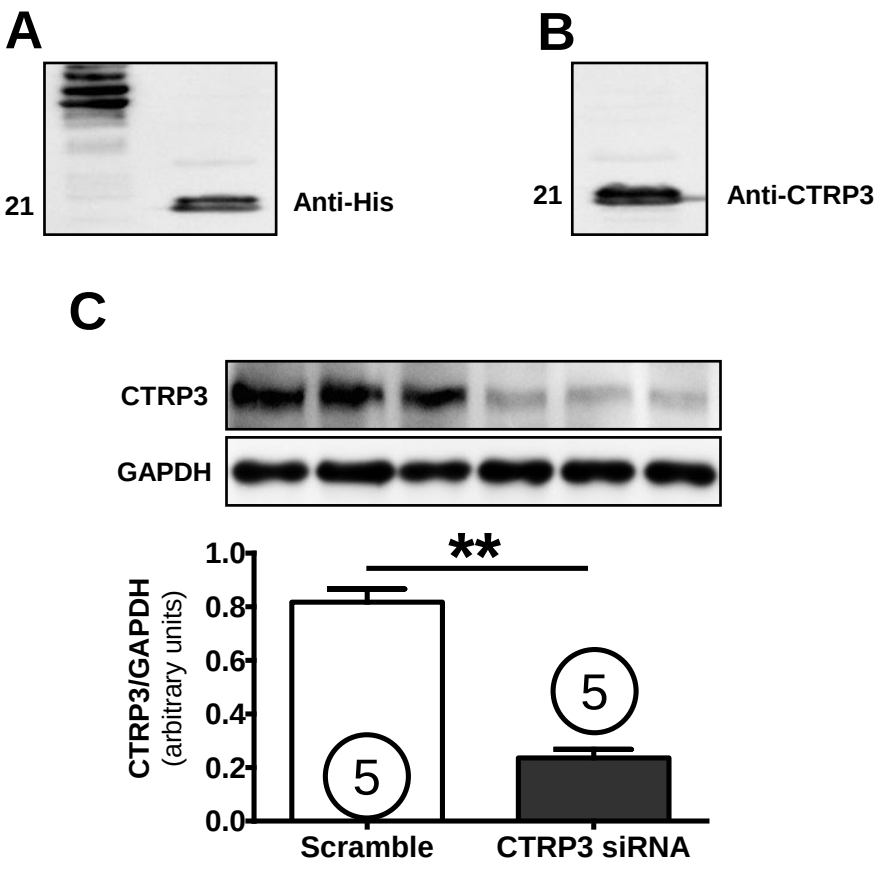

Figure S1. Western blots of recombinant globular domain of CTRP3 and CTRP3 SiRNA efficiency: Western blots of recombinant globular domain of CTRP3 with an antibody against His (A) or against CTRP3 (B); (C) Western blot of knock-down efficiency of mouse CTRP3 specific SiRNA. **P<0.01.

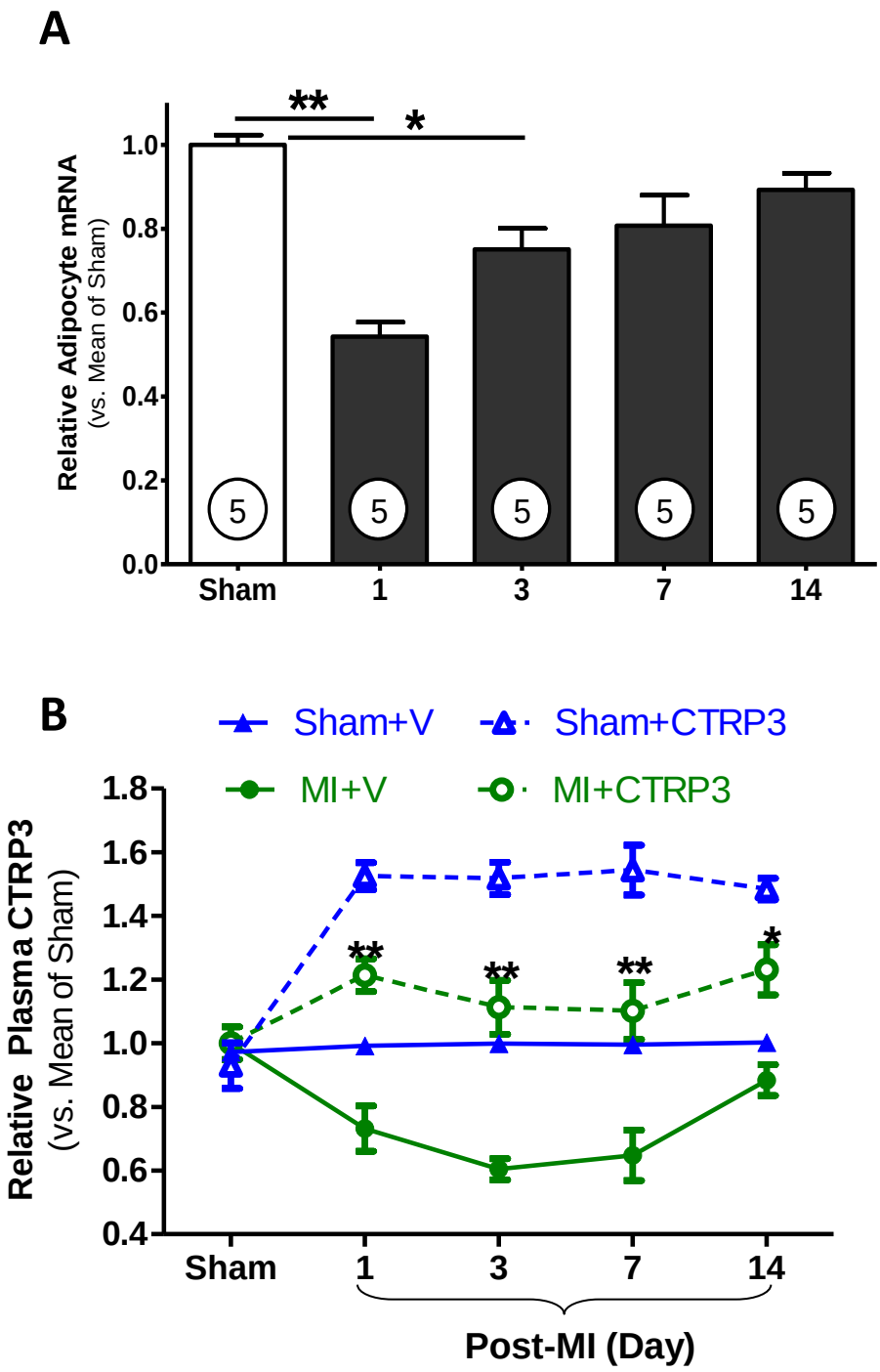

Figure S2. MI significantly inhibited adipocyte CTRP3 mRNA expression (A) and decreased serum CTRP3 levels (B, closed green circles connected with solid line). Supplementation of recombinant CTRP3 with an osmotic pump increased plasma CTRP3 levels in sham-operated mice (open blue triangles connected with dashed line) and prevented MI-induced serum CTRP3 reduction (open green circles connected with dash line). N=6/each time point; *P<0.05, **P<0.01 vs. MI+ Vehicle.

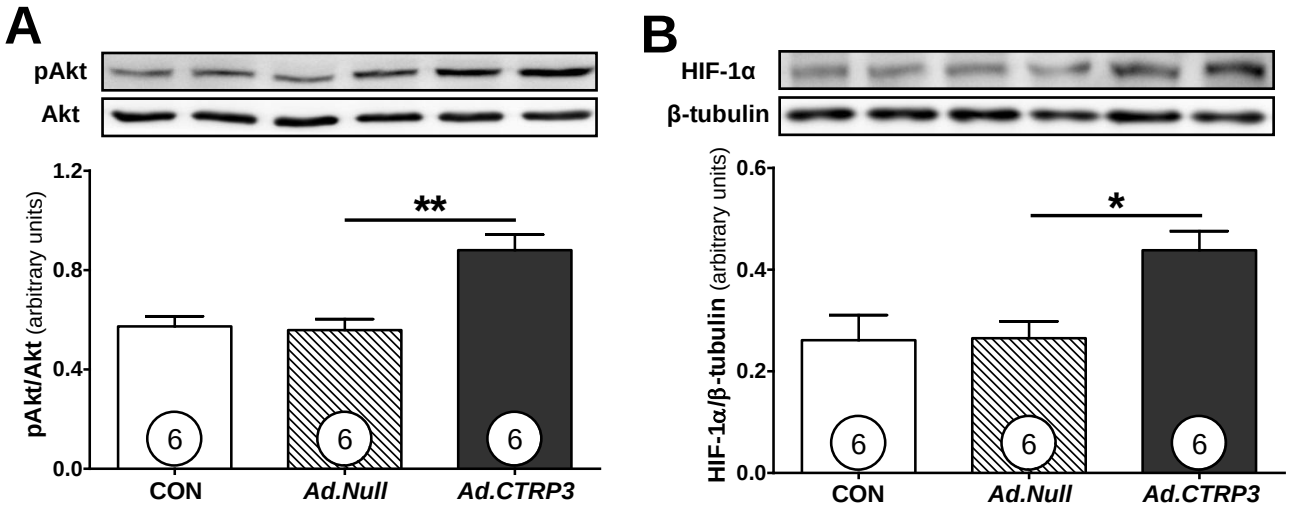

Figure S3. Adenoviral CTRP3 transfection of neonatal cardiomyocytes activated Akt (A) and upregulated HIF1α expression (B). Neonatal cardiomyocytes were transfected with Adenoviral CTRP3, and phospho-Akt (473)/Akt and HIF-1α/β-tubulin were determined 24 hours after transfection. *Ad.Null* had no significant effect upon Akt phosphorylation or HIF-1α production, but *Ad.CTRP3* significantly enhanced Akt phosphorylation, and increased HIF1-α expression. *P<0.05, **P<0.01.

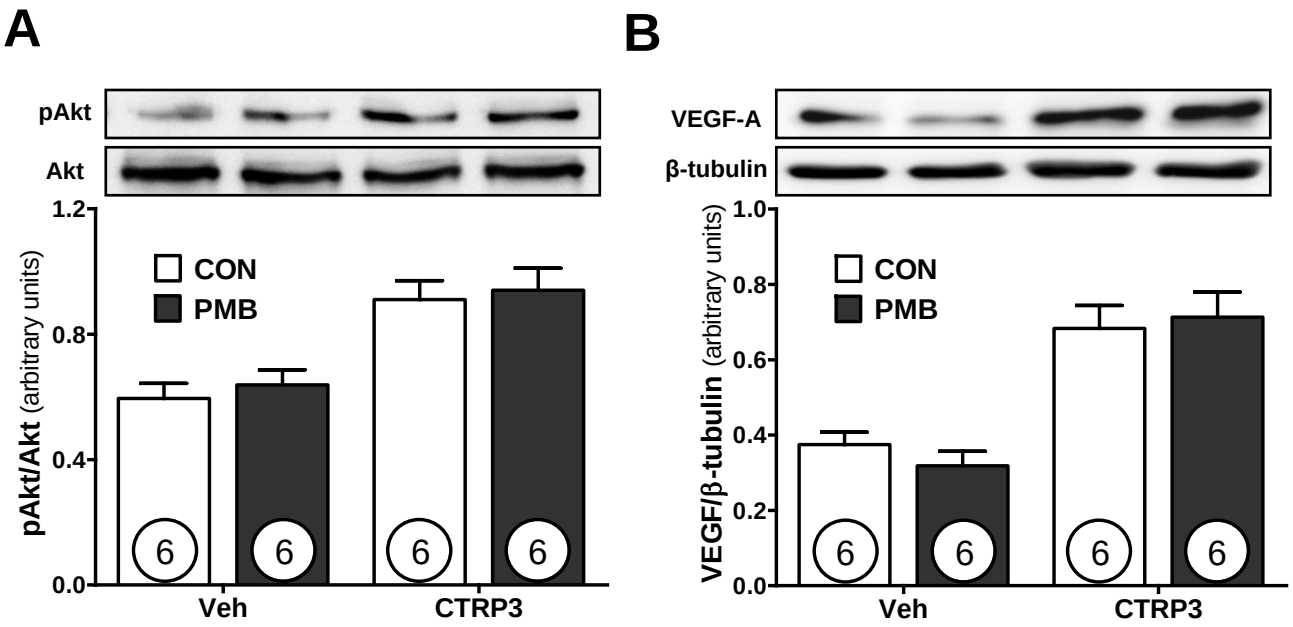

Figure S4. Cardiomyocyte Akt activation (A) and VEGF expression (B) in response to globular CTRP3 treatment were not blocked by polymixin B (PMB, 30μg/mL).

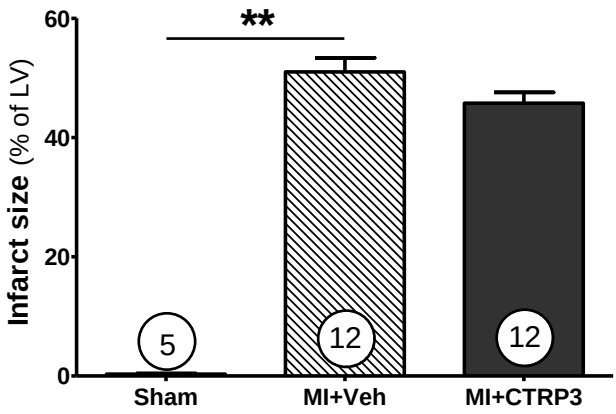

Figure S5. Treatment with CTRP slightly reduced infarct size. However, the difference is not statistically significant.